FORM PTO 1390
(REV 11-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: HARMSEN 002

U.S. APPLICATION NO. (If known, see 37 CFR 1 5): 09/554835

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATES	PRIORITY DATE CLAIMED
PCT/EP98/07389	18 November 1998	22 November 1997

TITLE OF INVENTION: USE OF THE E.COLI STRAIN DSM 6601 FOR TREATING DIARRHOEA IN VETERINARY MEDICINE

APPLICANT(S) FOR DO/EO/US: Hans PROPPERT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371 (f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371 (b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed.
5. [X] A copy of the International Application as filed (35 U.S.C. 371 (c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371 (c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). **(Unexecuted)**
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 & 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

Copy of International Application as published

EXPRESS MAIL LABEL NO. E: 479161776US
DATE: May 19, 2000

416 Rec'd PCT/PTO 19 MAY 2000

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/554835	PCT/EP98/07389	HARMSEN-002

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) – (5)):

		CALCULATIONS PTO USE ONLY
[ ]	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
[X]	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00	
[ ]	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $690.00	
[ ]	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
[ ]	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	1 - 20 =		x $18.00		
Independent claims	1 - 3 =		x $78.00		
MULTIPLE DEPENDENT CLAIM(s) (if applicable)			+ $260.00		
TOTAL OF ABOVE CALCULATIONS =				$840.00	
Reduction of ½ for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					
SUBTOTAL =				$840.00	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +					
TOTAL NATIONAL FEE =				$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31) + ($40.00 per property).					
TOTAL FEES ENCLOSED =				$840.00	
				Amount to be: Refunded	
				Charged	

a. [ ] A check in the amount of _____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 12-1095 in the amount of $840.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required or credit any overpayment to my Deposit Account No. 12-1095 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Use of the E.Coli Strain DSM 6601 for Treating Diarrhoea in Veterinary Medicine

The invention relates to the use of Escherichia coli strain DSM 6601 for prevention and treatment of diarrheas caused in animals by microbes.

Diarrhea in humans or animals is understood as frequent discharge of fluid feces for various causes, such as an allergic reaction to certain foods, but it suggests a reaction to microorganisms, especially bacteria, fungi or viruses or the toxins thereof. Because of great water and salt loss, prolonged diarrheas can lead to serious complications and even death. Severe diarrheas therefore always require treatment, especially in the case of young or already weakened humans or animals.

In a large proportion of cases, diarrheas are caused by bacteria that do not belong to the normal microorganisms of the flora of the affected body. Certain pathogenic strains of Escherichia coli, Salmonella and Shigella in particular play a major role in this regard. Diarrheas can be caused not only by bacteria, however, but also by infections with viruses, especially coronaviruses and rotaviruses, or by fungi, which in most cases are members of the Eumycota. Whereas diarrhea caused by bacteria can usually be combated very effectively now by administration of sorbents or nonsystemic sulfonamides or antibiotics, pharmaceuticals which can be used successfully in virus infections have not yet

been available, and hardly any pharmaceuticals are effective against colonization of the gastrointestinal tract with fungi.

Fungi are defined as a polyphylogenetic group of chlorophyll-free, heterotrophic and eukaryotic organisms, which can be single-celled or multiple-celled and whose reproduction proceeds with mitosis and meiosis, as for all eukaryotes, and by the formation of sexual or asexual spores or even by budding. The single-celled fungi that propagate only by budding are frequently grouped together as yeasts, even though the yeasts represent a conglomerate of different classes. Almost all Eumycota, or in other words the true fungi, pass through two or more morphologically distinguishable stages during their individual development, namely as teleomorphs, in which the spores are formed after meiosis, and one or more stages as anamorphs, in which spore formation is not associated with meiosis. Fungi whose teleomorphs are unknown or which have the lost the ability to form such are grouped together as Fungi imperfecti. Exact botanical classification is still unresolved for many of the fungi.

Because of their lack of chlorophyll, all fungi obtain their nutrition heterotrophically by degradation of organic substances; there are therefore saprophytes. In medicine a distinction is made between the opportunists, or in other words saprophytes which can become pathogenic rarely and only under strictly defined conditions. They include, for example, Candida and Aspergillus. Pathogenic saprophytes are exogenous organisms which live a normal saprophytic existence, but they must be regarded as pathogenic for humans or animals under all circumstances if an infection has developed. The fungi also

include obligate parasites, which cannot vegetate outside the host organism and which are found only in humans or animals; they include most dermatophytes.

One substantial difference between bacteria and fungi is based on the fact that fungi, in contrast to bacteria, are eukaryotes, meaning they possess a nucleus and membrane and, again in contrast to bacteria, they also possess mitochondria; another on the fact that the cell wall consists of chitin and/or cellulose, whereas the cell wall of the bacteria is formed from mucopeptides. This also explains why most antibiotics which are effective for bacteria fail for fungi, since in many known antibiotics the bacterial wall or the mitochondria are the main points of attack of these agents.

Some antibiotics are also known which can also be used for systemic and not merely topical treatment of fungal infections and which are characterized by a polyene structure, such as amphotericin B, griseofulvin, natamycin and nystatin. Synthetic systemic antimycotics are flucytosin and a series of azole derivatives such as ketoconazole and miconazole or fluconazole and itraconazole.

Whereas the exact mechanism of action of the polyene antibiotics is not yet known in all details, the synthetic azole derivatives act on the ergosterol synthesis in the cell membrane and thus influence the permeability of the cell wall.

A disadvantage of all antimycotics known heretofore, even those which can be used systemically, is that they usually act not as fungicides but merely as fungistats, thus necessitating a relatively long treatment period. Furthermore, cross resistances develop, both to the polyene antibiotics and to the azole compounds. In addition, finally, the price of these products is relatively high, thus hindering broad application in veterinary medicine.

There therefore continues to be a further need for veterinary pharmaceuticals which are capable effectively of combating diarrheas caused in animals substantially pathogenic fungi or even by the co-involvement thereof.

Completely surprisingly, it has now been discovered that such diarrheas can be effectively combated by using Escherichia coli strain DSM 6601, even when otherwise conventional medication with compounds having activity toward fungi has failed.

Escherichia coli, abbreviated hereinafter as E. coli, exists in numerous varieties, which differ as regards capsule antigens, surface antigens and flagella antigens and can therefore be subdivided into numerous serological types. Classification by serotypes, however, does not provide any indication of the different virulence of the pathogens. Representatives of one and the same serotype can have different pathogenic potential both in the human and in the animal body, ranging in the extreme case from avirulent to highly pathogenic. It is known, however, that E. coli strain DSM 6601 is rated as nonpathogenic to humans or animals. As an example, this strain is used in human medicine as a substitution preparation in infectious intestinal diseases due to Salmonella or Shigella, both in acute and

chronic cases. This E. coli strain is also used successfully in substitution therapy for other disorders of the intestinal flora, for example after antibiotic treatment or irradiation. It has not been unequivocally clarified whether what actually happens is that this special E. coli strain merely displaces the pathogenic bacteria strains, including the corresponding variants of E. coli or Proteus, thus causing a reduction in the toxins, or whether the metabolic products of E. coli strain DSM 6601 have a therapeutic effect in their own right.

Starting from these known explanations of the mechanism of action of living E. coli microorganisms, however, it could in no way be anticipated that treatment with these living cultures would have surprisingly extensive efficacy toward infection of the intestinal tract of animals with fungi, and in this context especially yeasts. Certainly apathogenic E. coli strains were occasionally in veterinary medicine in the fifties for diseases of cattle or pigs, sometimes also involving diarrheas, but the objective was therapy for nutritional disorders in piglets (Fischer, W., Experiences of a practicing veterinarian in the treatment of sick piglets from 1945 to 1950; Dissertation, Munich University 1950), or the treatment of Semper disease in cattle, which according to the results of this publication was obviously based on deficient nutrition due to the geology of the region (Häfele W., "Semper disease", a nutritional and developmental disorder of cattle in the Upper Black Forest in the vicinity of St. Blasien; Dissertation, Vet. Med. Animal Clinic, Munich University 1952). These early attempts to use certain coli strains experimentally for special diseases of pigs or cattle were entirely isolated, and not once did they provide the motivation to undertake further experiments of this type in diarrheas caused by bacteria. All the more surprising was the discovery that E. coli strain DSM 6601 exhibits a surprising effect even in

intestinal diseases caused exclusively or substantially by fungi, since fungal infections are particularly difficult to combat, especially when the mucous membranes of the intestine are affected, since the preparations used for bacterial infections have practically zero effect.

Heretofore surprisingly little has been known about the normal intestinal flora in various animal species, but infestation of the gastrointestinal tract with fungi and especially with yeasts must always be regarded as a pathological event.

The surprisingly rapid efficacy of the treatment with E. coli strain DSM 6601 suggests that the effect of this treatment does not depend or does not depend only on substitution of the fungal flora by a healthy bacterial flora, but instead that the strain contributes largely to an increase in the body's endogenous defense mechanisms, presumably because the metabolic products of this strain have a considerable immuno-stimulating effect.

The invention will be explained in more detail hereinafter by means of an example:

Example:

In a dairy farm in Saxony, which possessed on average a stock of 1480 milk cows, 600 heifers and young cows as well as 185 calves, the calf stock was characterized in early January 1997 by very good rearing results and low morbidity and mortality. Manifest gastric and intestinal diseases with diarrhea were extremely infrequent. Losses of calves were always below the 3% limit relative to the number of live-born calves.

By late January 1997 there was observed a sudden increase in the incidence of diarrheas among the stock, with clinically pronounced symptoms of gastroenteritis. The diseases began among the calves aged from seven to eleven days, were of various duration and were characterized by a morbidity of more than 90% and a mortality of more than 10%. The course of this disease was marked by the following symptoms: initially greenish-yellowish diarrhea with the consistency of thick paste, after which , in the further course of the disease, the feces became slimy, increasingly thin and mostly watery. The body temperature readings were slightly elevated at the beginning of the disease (39.7 to 39.9 °C), but then dropped rapidly in the further course and reached only still lower limits, some in the range of 37.8 to 37.5 °C. The diseased calves exhibited pronounced aversion to suckling and increasing weakness, ultimately just lying down in one place in the further course of the disease. Seriously sick animals had to be forcibly fed.

After an illness duration of three to nine days, the losses became increasingly worse. The autopsies performed consistently revealed the most prominent

findings, namely a fluid, flocculent to slightly bloody intestinal content and advanced, ulcerative inflammations of the omasum and abomasum. In the month of April 1997, the incidence of the disease reached values of almost 100% and the mortality rose to more than 15%.

The microbiological studies of the gastric and intestinal contents and of the internal organs were initially without specific results until February 1997, when yeasts of the genus Candida (C. glabrata and C. albicans) were isolated from the gastric and intestinal contents of two dead calves. Intestinally pathogenic E. coli were not found. These yeasts were detected in all further autopsies of dead calves and in the studies of fecal specimens of calves and their mothers. Further specific studies of fecal and colostric specimens of the cows and heifers yielded identical results. Supplementary studies for viruses, namely coronaviruses and rotaviruses, as well as for cryptosporidae revealed coronavirus particles only in two cases and rotaviruses in one case among dead calves.

The origin of the yeasts remained inexplicable for some time, until March 1997, when a study of brewery residues used as fodder was able to detect yeasts (with Candida glabrata dominating) in a concentration of 3.6×10^7 CFU per g of fodder.

The therapeutic measures immediately performed extensively, such as dietary drinks, antiphlogistics, astringents, electrolytes and cardiovascular agents led only to unsatisfactory results. When sulfonamides and antibiotics were used, the animals died six to eight hours after application. Once these therapies had proved unsuccessful, therapeutics containing humic acids as the active agents

were used. Even these actions did not achieve any perceptible improvements of the occurrence of the disease.

Of the 236 calves born alive in two months from 24 April to 23 June 1997, all animals became sick and 41 died in this period, corresponding to a proportion of 17.4%. Of those, 29 animals, corresponding to 70.7% of the losses, died from diarrheas; eight animals, corresponding to 19.5% of the losses, died from diarrheas with concomitant bronchopneumonia; and four animals, corresponding to 9.8% of the losses, died from other causes.

In the period from 25 June 1997 to 04 September 1997, a suspension of living E. coli strain DSM 6601 was then administered to 300 newborn calves in an amount of 15.0 ml per calf per day – corresponding to ... CFU/ml. This dose was independent of the weight and age of the animal and was administered orally, specifically for a duration of 10 to 13 days after birth. In the period from 24 June to 08 July 1997, 61 calves were treated in this way. Two exhibited diarrhea, corresponding to a morbidity of 3.3%. None of the animals died of this disease.

In the period from 09 July to 23 July 1997, 49 calves were treated correspondingly. None of these suffered from gastroenteritis with diarrhea. The mortality due to this disease was therefore 0%.

In the period from 23 July to 06 August 1997, 64 calves were treated. Two exhibited diarrhea, corresponding to a morbidity of 3.1%. None of the animals died of this disease, and so for this period the mortality was equal to 0.

After all existing therapeutic options had been exhausted, the occurrence of diarrheas due to gastroenteritides in suckling calves was prevented almost completely by the preventive and therapeutic application of a suspension of E. coli strain DSM 6601.

It was possible to considerably reduce the use of other pharmaceuticals and dietetics, and so the costs expended for these substances were lowered by 70%.

Claims

1. The use of Escherichia coli strain DSM 6601 for synthesizing pharmaceuticals for prevention and treatment of diarrheas caused in animals by microbes, with involvement of pathogenic fungi.

ABSTRACT

The invention relates to the use of the Escherichia coli strain DSM 6601 to produce medicaments for preventing and treating microbially-caused diarrhoea involving pathogenic fungi in animals.

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

ATTORNEY'S DOCKET NO.: HARMSEN 3.3-002

As a below-named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF THE E.COLI STRAIN DSM 6601 FOR TREATING DIARRHOEA IN VETERINARY MEDICINE the specification of which

☐ is attached hereto

☒ was filed on November 18, 1998 as United States Application Number or PCT International Application Number PCT/EP98/07389 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)			
COUNTRY	**APPLICATION NUMBER**	**DATE OF FILING (month, day, year)**	**PRIORITY CLAIMED**
Germany	197 51 907.5	November 22, 1997	YES ☒ NO ☐
			YES ☐ NO ☐
			YES ☐ NO ☐
LISTING OF FOREIGN APPLICATIONS CONTINUED ON PAGE 3 HEREOF ☐ YES ☒ NO			

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Application Number: Filing Date:

Application Number: Filing Date:

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Parent Application Serial Number: Parent Filing Date: Parent Patent No.:

U.S. Parent Application Serial Number: Parent Filing Date: Parent Patent No.:

PCT Parent Number: Parent Filing Date:

LISTING OF US APPLICATIONS CONTINUED ON PAGE 3 HEREOF: ☐ YES ☒ NO

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Customer Number 000530

DIRECT ALL CORRESPONDENCE TO: Customer No. 000530

DECLARATION -- Page 2

ATTORNEY DOCKET NO. HARMSEN 3.3-002

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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Second Inventor's signature _____ **Date** _____

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Third Inventor's signature _____ **Date** _____

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Sixth Inventor's signature _____ **Date** _____

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Full name of seventh joint inventor, if any (given name, family name):

Seventh Inventor's signature _____ **Date** _____

Residence: Citizenship:

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Full name of eighth joint inventor, if any (given name, family name):

Eighth Inventor's signature _____ **Date** _____

Residence: Citizenship:

Post Office Address:

☐ Additional inventors are being named on separately numbered sheets attached hereto.